

(19)



Europäisches Patentamt

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(11)

EP 0 731 108 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:

11.09.1996 Bulletin 1996/37

(51) Int. Cl.<sup>6</sup>: C07K 14/48

(21) Application number: 95103458.6

(22) Date of filing: 10.03.1995

(84) Designated Contracting States:  
DE

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(54) Method of manufacturing a therapeutic agent for the regeneration of oligodendrocytes

(57) With the method of preparing a therapeutic agent for the treatment of multiple sclerosis, wherein human  $\beta$  nerve growth factor (NGF) is brought into a pharmaceutically acceptable formulation for administering in a dose between 0.05  $\mu$ g and 5  $\mu$ g/kg body weight in 5 to 10 injections at an interval of 7 to 21 days, the regeneration of oligodendrocytes, especially for the treatment of multiple sclerosis, is improved and remyelination is accelerated.

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**Description**

The present invention concerns a novel method for the regeneration of oligodendrocytes, in particular, of human oligodendrocytes, and for the treatment of diseases in which a demyelination of nerve fibers occurs, a method of manufacturing a therapeutic agent, as well as the use of human  $\beta$  nerve growth factor (NGF) for said therapeutic use.

The covering of nerve fibers in the central nervous system (CNS) with myelin is essential for the function of neuronal signal transmission. The myelin sheath is formed by oligodendrocytes (OL), the fibers of which wrap around the axon of a nerve cell. Demyelinating diseases such as multiple sclerosis in which the myelin sheath of the axon is damaged or destroyed also leads to impairments of the OL. However, the OL remains capable of regenerating the myelin sheaths. Therefore, the identification and characterization of factors which are responsible for increased regeneration of OL is very important for the molecular understanding of demyelination diseases, such as multiple sclerosis (MS), and for the development of therapeutic agents.

It is known from PCT/EP92/01173 (17) that the regeneration of oligodendrocytes is improved when they are treated with NGF or active fragments of NGF.

NGF is a neurotrophic factor which is well characterized. Its gene is described in Ullrich et al. (1993) (1) and EP-B 0 121 338 (US-P 5,169,762) (13). The recombinant production of NGF from E.coli is described in EP-A 0 544 293 (15), EP-A 0 450 386 (US-P 5,235,043) (14), EP-A 0 370 171 (16) and US Serial No. 08/266610 (18).

NGF has potent and beneficial effects on cholinergic neurons after axotomy (see, for example, Hoffman et al. (1990) (2)). NGF infusions stimulate the regeneration of transected cholinergic neurons (Gage (1988) (3), Tuszyński et al. (1990) (4)). It is further known that infusions of NGF stimulate the expression of choline acetyltransferase (ChAT) (Hefti et al. (1984) (5) and p75NGF receptor mRNA (Gage et al. (1989) (6).

For the therapeutic application, NGF is infused for a period of several weeks. Olson et al. (1991) (11) describes an infusion of NGF through an intraventricular cannula for 23 days with a total dose of 3.3 mg (corresponding to approximately 140  $\mu$ g/24 hr) for the treatment of Parkinson's patients after having received fetal dopaminergic grafts. It is also known to deliver NGF therapeutically in Alzheimer's disease via a programmable pump planted subcutaneously into the abdominal wall and connected by a subcutaneous catheter to the intraventricular catheter. To the patient a total of 6.6 mg of NGF was delivered during three months at a rate of 15  $\mu$ l/hr (corresponding to approximately 75  $\mu$ g/24 hr (Olson et al. (1992) (12))).

**Summary of the Invention**

It was surprisingly found that for the regeneration of oligodendrocytes, i.e. in MS, an interval application leads to an improved and accelerated remyelination of damaged nerve fibers as compared to continuous application of the therapeutic agent.

The invention concerns a method of preparing a therapeutic agent for the treatment of multiple sclerosis, 10 the method being characterized in that human  $\beta$  nerve growth factor (NGF) is brought into a pharmaceutically acceptable formulation for administering in a dose between 0.05  $\mu$ g and 5  $\mu$ g/kg body weight in 5 to 10 bolus injections as an interval of 7 to 21 days.

According to the invention, NGF is administered in a dose between 0.05  $\mu$ g and 5  $\mu$ g/kg, 3.5 to 350  $\mu$ g per injection, at an interval of 7 to 21 days. Preferably, the dose is applied in 5 to 10 injections.

In a preferred embodiment of the invention, it is preferred to use 0.1  $\mu$ g to 1  $\mu$ g/kg.

It is preferred to administer NGF intrathecally, into the cerebrospinal fluid space of the ventricle or spinal cord, preferably of the lateral ventricle or the lumbar spinal cord.

**Detailed description of the invention**

The term "NGF" means  $\beta$ -unit of human NGF.  $\beta$ -NGF has an amino acid sequence of 118 amino acids and is present as a dimer in solution. The amino acid and DNA sequence is described in Ullrich et al. (1993) (1).

The pharmaceutical compositions which are used according to the invention and contain  $\beta$ -NGF may be 35 administered in any sterile biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline or dextrose-solution. The amount of NGF protein which will be effective in the treatment of MS is in a dose between 0.05  $\mu$ g and 5  $\mu$ g/kg body weight at an interval of 7 to 21 days, preferably in 5 to 10 injections. It is further preferred to use 3.5 to 350  $\mu$ g/injection.

Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal administration.

45 In addition, it may be desirable to introduce the pharmaceutical composition of the invention into the central nervous system by any suitable route, including intrathecal, e.g. intraventricular injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example attached to a reservoir, such as an implantable port catheter system, such as the Periplant<sup>®</sup> filtrosafe (B. Braun GmbH, Spangenberg, DE).

Furthermore, it may be desirable to administer the pharmaceutical compositions, which are used according to the invention, locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, injection by means of a catheter or by means of an implant, said implant being of a porous,

non-porous or gelatinous material, including membranes, such as sialastic membranes or fibers.

The following examples are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

#### **Example 1**

##### **Experimental design of an animal model of demyelination**

###### Lesion:

An experimental model of demyelination was established in the female, adult "Göttingen Mini Pig" (age: 10-14 months, weight: 25-30 kg) by stereotaxic injection of lysolecithin (LL) at multiple sites into the periventricular subcortical white matter of the brain (usually 2-3 injection sites located in one hemisphere; 5 µl of 1% LL in 0.9% saline per lesion infused over a period of 15 min). This infusion causes a rapid reduction of myelin sheaths within the diffusion area of the detergent (Blakemore (1978) (7)).

###### NGF-application:

Immediately after LL application, a catheter system (Periplant Filrosafe®, Braun, Melsungen, Germany) is implanted either into the lateral ventricle of the brain or in the subarachnoidal space of the lumbar spinal cord (Motsch and Robert (1987) (8); Krames and Lanning (1993) (9)). Two ways of NGF application were used:

1) After fixation of the catheter with Ionoce® bone cement (Ionom, Seefeld, Germany) the intrathecal catheter is connected with a subcutaneous drug delivery system (Periplant Filrosafe®, Braun, Melsungen, Germany) which is implanted subcutaneously in the upper back region. NGF (dissolved in phosphate-buffered saline, pH 7.2) injected every 2nd day through the skin into the port, starting 2 days after initial LL lesions, by single bolus injections into the port at a dose of 0.05-5 µg/kg body weight (3.5-350 µg per injection). 3-5 injections were carried out into the cerebrospinal fluid within a period of 6-12 days after LL-induced demyelination.

2) The effect of single bolus injections of NGF described above was compared with the continuous infusion of NGF into the lateral ventricle via osmotic minipumps (Alzet® for 6-12 days at a dose of 0.5 µg/h).

###### Controls:

5 Controls were carried out by replacement of NGF solution with an equal dose of Cytochrome C applied in the same ways and time interval as described in 1) and 2).

###### Evaluation of NGF effects:

10 At the end of the NGF (control, respectively) application period, the experimental animals were sacrificed, the brain was removed and prepared for histological examination: The areas of interest were studied with routine histological (H.E. staining, Luxol Fast Blue staining), immunocytochemical (myelin basic protein - MBP) and electronmicroscopic (myelin lamellae) procedures.

###### Findings:

20 — Injection of LL causes a widespread demyelination throughout the area of diffusion.

— Chronic infusion of infusion of NGF via Alzet pumps:

25 An improved and accelerated remyelination of the lesioned myelin sheaths of nerve fibers was detected within the application interval as compared to controls. Significant regeneration was observed as early as 7 days after initial lesion. After 12 days an almost complete regeneration was detected in NGF-treated animals, whereas a significant demyelination was still present in the white matter of sham-treated animals.

30 — Bolus NGF injection every 2nd day:  
Significant regeneration was also noted under NGF treatment in this system, with no apparent differences to animals receiving chronic infusions of NGF. Furthermore, NGF injected in the CSF space in the area of the spinal cord showed the same positive effect on remyelination as found for intraventricular injections.

35 The data demonstrate that injections of NGF into the cerebrospinal fluid lead to an improved and accelerated remyelination, most likely due to regeneration and proliferation of oligodendrocytes. Compared to chronic NGF infusion with the help of pumps, the application via an intrathecally implanted port allows an easy-to-handle, save and inexpensive way of supplying the brain with the neurotrophin at an adjustable and optimal concentration for maximal therapeutic efficacy. In addition, in patients with multiple sclerosis suffering from repeated attacks (i.e. relapsing-remitting MS) immediate interval treatments at the time of the attack within a period of several years are possible, minimizing the risk of complications (i.e. infections) due to necessary changes of catheter systems or refills of pumps needed during chronic or repeated infusions.

Additional supporting data:

- There is a clear correlation between the degree of remyelination and functional improvements in the state of demyelinating diseases (IFN $\beta$  study group (1993) (10)).
- Data obtained from animal models using pigs have a great clinical relevance in biomedical research, i.e. numerous studies on the cardiovascular system, skin and peripheral nervous system that have proven that data from porcine tissue are applicable on human subjects.

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4. The method according to claims 1 to 3 wherein human  $\beta$  nerve growth factor is administered into the cerebrospinal fluid space of the ventricle or in the spinal cord.

5. The method according to claim 4 wherein the intraventricular injection is carried out via an intraventricular catheter attached to an implanted port for injection.

**List of References**

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 (14) EP-A 0 450 386 (US-P 5,235,043)  
 (15) EP-A 0 544 293  
 (16) EP-A 0 370 171  
 (17) PCT/EP92/01173  
 (18) US Serial No. 08/266610

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**Claims**

1. A method of preparing a therapeutic agent for the treatment of multiple sclerosis wherein human  $\beta$  nerve growth factor (NGF) is brought into a pharmaceutically acceptable formulation for administering in a dose between 0.05  $\mu$ g and 5  $\mu$ g/kg body weight in 5 to 10 injections at an interval of 7 to 21 days.

2. The method according to claim 1 wherein human  $\beta$  nerve growth factor is administered in a dose between 0.1  $\mu$ g and 1  $\mu$ g/kg body weight.

3. The method according to claim 1 or 2 wherein human  $\beta$  nerve growth factor is administered in 3.5-350  $\mu$ g/injection.

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## EUROPEAN SEARCH REPORT

Application Number  
EP 95 10 3458

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X,D	DE-A-41 25 933 (MAX-PLANCK-GESELLSCHAFT....) * claims 1-14 * * page 3, line 8 - line 17 * ---	1-5	C07K14/48
X	EP-A-0 351 808 (G. D. SEARLE & CO.) * claims 1-54 * ---	1-5	
A	ANNALS OF NEUROLOGY, vol. 24, no. 1, July 1988 USA, pages 64-72, GEN SOBUE ET AL * abstract *	1-5	
A,D	ARCHIVES OF NEUROLOGY, vol. 48, no. 4, April 1991 USA, pages 373-381, L. OLSON ET AL * abstract *	1-5	
A,D	JOURNAL OF NEURAL TRANSMISSION, vol. 4, no. 1, 1992 AUSTRIA, pages 79-95, L. OLSON ET AL * abstract * * page 81, last paragraph *	1-5	TECHNICAL FIELDS SEARCHED (Int.Cl.6)  C07K A61K
<p>The present search report has been drawn up for all claims</p>			
Place of search  BERLIN	Date of completion of the search  11 August 1995	Examiner  Siatou, E	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			